Drug resistance mutations in HIV

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Drug resistance mutations in HIV: new bioinformatics approaches and challenges

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Drug resistance mutations appear in HIV under treatment pressure. Resistant variants can be transmitted to treatment-naive individuals, which can lead to rapid virological failure and can limit treatment options. Consequently, quantifying the prevalence, emergence and transmission of drug resistance is critical to effectively treating patients and to shape health policies. We review recent bioinformatics developments and in particular describe: (1) the machine learning approaches intended to predict and explain the level of resistance of HIV variants from their sequence data; (2) the phylogenetic methods used to survey the emergence and dynamics of resistant HIV transmission clusters; (3) the impact of deep sequencing in studying within-host and between-host genetic diversity of HIV variants, notably regarding minority resistant variants.

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Introduction

Drug resistance mutations (DRMs) arise in Human Immunodeficiency Virus-1 (HIV-1) due to antiretroviral treatment pressure, leading to viral rebound and treatment failure [1,2]. Furthermore, drug-resistant HIV variants can be transmitted to treatment-naive individuals and further spread throughout the population over time [3–5]. These transmitted drug-resistant (TDR) HIV variants limit treatment options and have clinical and public health implications worldwide. The scale of TDR varies globally; in the US and Europe, the prevalence of TDR has decreased or stabilized at between 5% and 15% [6–11]. However, in resource-limited countries, the prevalence of TDR is becoming a pressing health issue [11,12], with many regions reporting an exponential increase in prevalence and many surpassing 10% prevalence [13]. Indeed, WHO have suggested that if the prevalence of TDR exceeds 10% in a country, then first-line regimens should be reconsidered [14]. Because of this, a number of countries in Africa and Asia have revised their national treatment guidelines [12].

There are five main classes of HIV-1 antiretroviral therapies, which target different virus proteins: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), (ii) non-nucleoside reverse transcriptase inhibitor (NNRTIs), (iii) protease inhibitors (PIs), (iv) integrase inhibitors (INIs or INSTIs) and (v) entry inhibitors. The reverse transcriptase, protease and integrase proteins are encoded in the *pol* gene, while the entry inhibitors induce DRMs in the *env* gene. NRTIs and NNRTIs date back to the 80s and are currently the most commonly used drugs. PIs and INIs appeared more recently, in the mid-90s, and are still in development [15,16]. PIs and INIs are associated with lower levels of resistance compared to reverse transcriptase-based therapy. INIs are increasingly used in first-line regimens in the presence of NNRTI resistance at the population level [12]. In total, there are ~25 available drugs, all of which are associated with known DRMs. A list of DRMs is regularly updated [17•] by a consortium of international experts, who select and classify the DRMs to be surveilled (~175 in total) based on genotype analyses, phenotypic resistance tests and clinical outcome in patients on antiretroviral therapies. Primary DRMs directly confer resistance to treatments, but some mutations have an accessory role, increasing drug resistance when appearing alongside primary DRMs, while others seem to have a compensatory role and reduce the fitness cost for primary DRMs. All this, combined with the development of new antiretroviral drugs [16,18•] and the use of antiretroviral treatments in high-risk populations by pre-exposure prophylaxis [19,20], makes it particularly important to further our understanding of

HIV adaptation, detect new mutations associated with drug resistance, and survey the emergence of resistant-HIV transmission clusters in infected populations, especially in low-income countries.

For all these endeavors to advance, bioinformatics methods and large well-curated sequence databases are essential. The Stanford HIV Drug Resistance Database (https://hivdb.stanford.edu/) is the largest public repository and most widely used online resource for HIV drug resistance. It currently comprises: (1) ~450 000 sequences (reverse transcriptase, protease or integrase) from ~200 000 patients with treatment status, from all around the word; (2) ~60 000 results of drug susceptibility assays from HIV-1 virus isolates; (3) clinical outcome data from 15 clinical trials; (4) many software programs and web services to query this data. Several countries and regions have set up national databases of HIV sequences generated through routine resistance genotyping. These repositories link genotypic data with anonymized clinical and demographic information, and are regularly updated, making these national databases an attractive resource to study and monitor drug resistance. However, due to the sensitive nature of patient-derived information, the content of these national databases is non-public and only available on request. The main national/regional HIV drug resistance repositories include: (i) The UK HIV Drug Resistance Database (https://www.hivrdb.org.uk/), which is the central repository for resistance tests performed as part of routine clinical care throughout the UK since 2001. It currently comprises over 165 000 test results, most in the form of annotated *pol* gene sequences and includes over 60% of the newly diagnosed patients in the UK, with linked clinical data available for the majority of patients. (ii) The Swiss HIV Cohort Study Drug Resistance Database (http://www.shcs.ch/) that includes data and meta-data from over 80% of new diagnoses in Switzerland. (iii) The PANGEA database [21•] with data from sub-Saharan Africa, a radically different region where the pandemic started and is of great concern, which holds over 12 000 nearly complete HIV-1 genomes, with basic-to-extensive associated epidemiological metadata.

In the following, we describe the main approaches to decipher this data, and the potential of Next Generation Sequencing (NGS) to better understand and survey the emergence of DRMs and their transmission.

Machine learning approaches to study and predict resistance

The presence of DRMs before the start of an antiretroviral therapy regimen is a strong predictor of the success or failure of that regimen. Resistance testing using DNA sequencing is performed routinely in upper-income countries, and with increasing frequency in low-income and middle-income countries. To this end, computer programs are used to analyze the virus sequence of the patient (i.e. the virus genotype) and predict the level of resistance of this sequence to available drugs (i.e. the resistance phenotype of the virus). Computer programs can also be used to optimize the combination of multiple drugs [22].

The standard approach to predict the level of resistance of HIV sequences (either in the reverse transcriptase, protease or integrase proteins) is to rely on known resistance mutations to various antiretroviral therapies. HIVdb [23] uses expert rules to combine mutations (primary or accessory) observed in the studied sequences, while WebPSSM [24] uses position-specific scoring matrices. However, machine learning methods are increasingly used for this purpose, often via web services [25,26]. These methods first learn a statistical model from a set of training examples, that is, virus sequences and their resistance level measured experimentally using PhenoSense assays [27], and then assess the accuracy of the learned model using an independent set of testing examples or a cross-validation procedure. We distinguish classification methods, which predict the effectiveness of a given antiretroviral therapy [28•], and regression approaches, which predict the fold resistance ratio of the given sequence compared to the wild type [29]. Initial approaches were based on decision trees [30], support vector machines [25], logistic regression [31] and neural networks [29]. The latter showed higher accuracy (on average ~85%) than the rule-based methods used, for instance, by HIVdb (~70%) [29].

Deep learning models (i.e. neural networks with complex architectures and a large number of hidden neurons [32]) are a major focus in current machine learning research and have been successfully applied to many biological problems [33]. Moreover, recent methods make it possible to map model outputs back to subsets of the most influential input features [34]. This approach was explored by Steiner *et al.* [28•], who evaluated the performance of three deep learning architectures (multilayer perceptron, bidirectional recurrent neural network, and convolutional neural network) for drug resistance prediction using genotype-phenotype data available from HIVdb, as training and testing data (via cross-validation). The resistance to 18 antiretroviral therapies was learned from ~2100 sequences associated with PI susceptibility, ~1800 sequences associated with NNRTI susceptibility, and 2100 sequences associated with NRTI susceptibility (measured by PhenoSense assays [27], as for PI and NNRTI data). The accuracy of convolutional neural networks ranged from 86% to 96% and a large number of known DRMs were among the most influential input features. Authors suggest that other influential mutations could also be associated with resistance. These findings underscore the gain in accuracy brought by machine learning approaches, compared to rule-based methods (e.g. HIVdb). However, the main limitation is the low

number of available sequences with drug susceptibility measurement given that deep learning is commonly used with much larger data sets (>10 000 and frequently >100 000 training examples).

Another approach was explored in Ref. [35•] to study resistance patterns, epistasis and discover new DRMs using: (i) A much larger reverse transcriptase sequence dataset (∼55 000 sequences) for training; (ii) A classification task to discriminate treatment-naïve from treatment-experienced sequences; (iii) Simpler machine learning models, such as random forest and logistic regression; (iv) Testing on a very different African dataset with subtypes not seen in the training data to improve robustness and to limit the impact of phylogenetic confounding factors. These choices were made with one goal in mind: interpretability, because it allows the easy extraction of mutations associated with resistance from important (influential) classifier features. To summarize, more DRMs are expected among treated patients than among naïve ones, even if we expect some DRMs to be present among naïve patients due to TDRs. To extract DRMs we can then perform tests (e.g. exact Fisher tests [36]) or use more advanced, interpretable machine learning methods [35•]. To confirm and further explore the nature of newly discovered resistance associated mutations, the training process was repeated after removing features and sequences corresponding to known DRMs (Figure 1a). This approach allowed the finding of six new potential accessory mutations. Two of these are L228H and L228R (i.e. mutations from L to H and L to R, respectively, at reference position 228 of reverse transcriptase), which are spatially very close to both the active and regulatory sites of reverse transcriptase (Figure 1b), and are overrepresented in sequences containing known DRMs (Figure 1c.1 and c.2).

Phylogenetic methods to decipher the spread of resistance

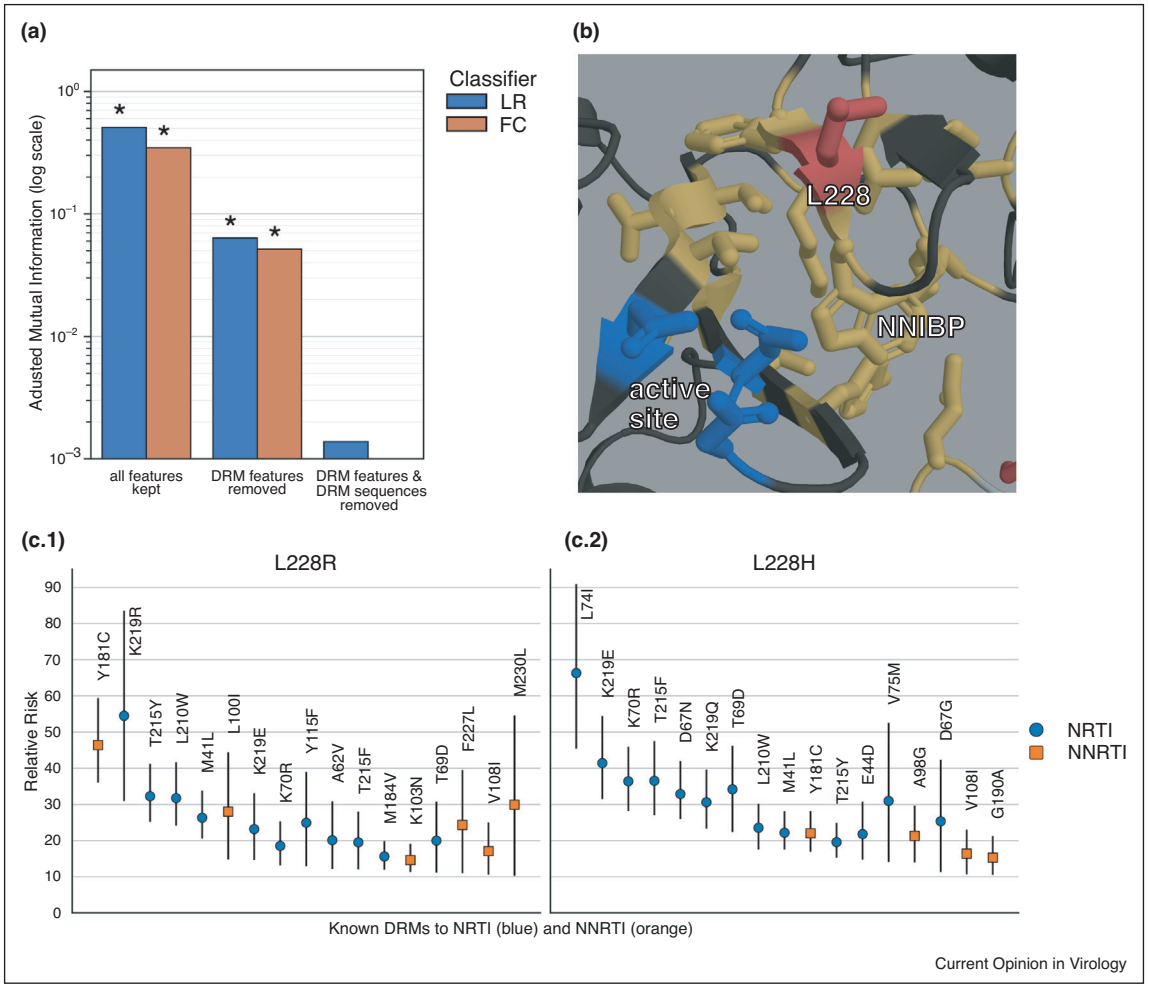
Following acquisition under treatment pressure, DRMs and resistance-associated mutations can be transmitted to treatment naïve patients. We distinguish acquired and transmitted drug resistances (TDRs). TDRs can be further separated into those corresponding to treated-to-naïve versus naïve-to-naïve transmissions. The latter are particularly problematic, as they can cause the emergence of resistance clusters in the naïve population. On the other hand, DRMs have some fitness cost and in the absence of treatment they tend to be reverted to the wild type amino acid. Some DRMs have been shown to revert rapidly (e.g. M184V in reverse transcriptase, associated to NRTIs [37,38]), while others have a low fitness cost (e.g. L90M in protease [39]) and tend to induce large resistance clusters ([40]; Figure 2).

Traditionally, for routine resistance genotyping a unique consensus virus sequence per patient is used to characterize the variants infecting a given individual, and phylogenetic trees are inferred from these consensus sequences to study the emergence, transmission and reversion of DRMs at the population level. In these trees, sequences that cluster together represent transmission clusters and each of the internal tree nodes corresponds to a transmission event. However, with one sequence per patient one cannot infer the direction of transmission, that is, distinguish the transmitter and recipient partners corresponding to a given tree node. With multiple sequences per patient, as obtained from NGS, phylogenetic methods help to infer the most likely transmission history [41]. However, reliably identifying the direction of transmissions remains challenging [42] and depends on, among other factors, the genetic diversity captured in the virus sequences of the individuals [43]. To summarize, the genetic diversity of the virus is expected to be significantly higher for the transmitter than for the recipient, but both can be similar, for example, when the infection dates are close. Moreover, one can never rule out the possibility of an intermediate, unsampled individual. Despite these limitations, phylogenetic inference has proved a promising tool for the population-level analysis of HIV resistance transmission. For example, phylogenetic tools are key in the PANGEA project [21•] to analyze the source-sink dynamics in several Sub-Sahara African settings, aiming to find generalizable characteristics of transmitters and transmission events, and guide recommendations for HIV treatment and prevention policies.

To decipher DRM transmissions, the most likely transmission clusters are extracted from the phylogeny. Genetic clusters correspond to well-supported subtrees that contain sequences closely related to each other and distant from the rest of the tree based on user-defined genetic thresholds [45]. A genetic cluster can be interpreted as representing a recent outbreak, for example, when a virus acquires a DRM and the patient starts transmitting the resistant virus. If most of the individuals in this cluster contain the same DRM, they form a resistance cluster, from which the number of within-cluster naïve-to-naïve TDRs can be estimated. This approach was used to study TDRs in Switzerland [46,47], Denmark [48], Ethiopia [49] and the USA [50].

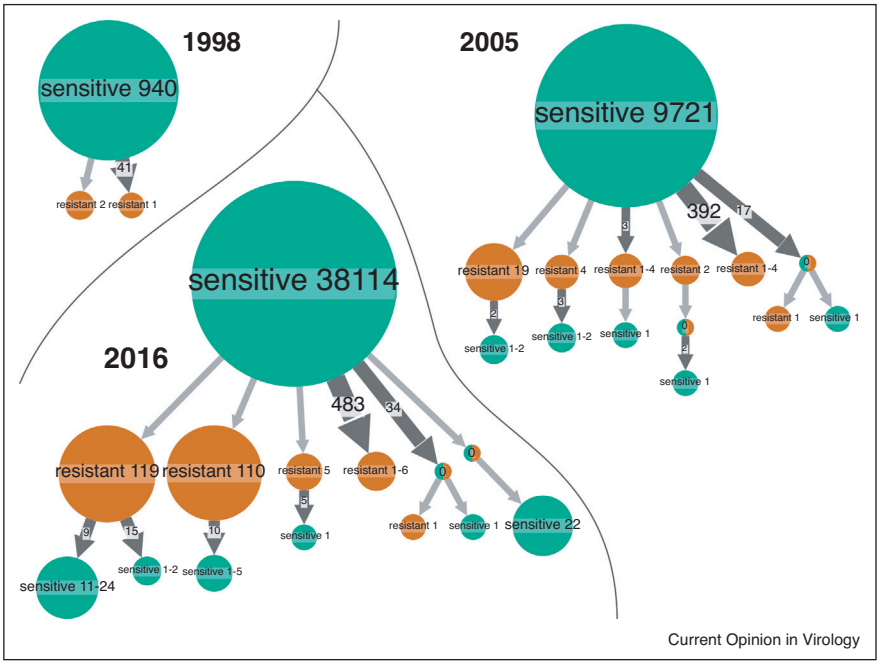
The second approach refines the previous one by using ancestral state reconstruction of a binary character describing the presence/absence of the studied DRM. Tree tips are annotated using the presence or absence of mutations, and the internal node states are inferred using parsimony [4] or maximum-likelihood [51•] methods. The clusters are defined by subsets of tips and nodes, all of which have the same resistance status and descend from a unique node corresponding to the first within-cluster transmission. Isolated, resistant tips with treatment-experienced status are interpreted as acquired drug

Figure 1



Detecting resistance associated mutations using machine learning.
This figure is adapted from Ref. [35•]. The authors used a large UK dataset (n = 55 539) of reverse transcriptase sequences from HIV patients, who have received treatment or not [44]. Sequences from this dataset were encoded as binary vectors where each feature corresponded to a specific mutation. These vectors were used to train classifiers, logistic regression (LR) and a Fisher-test-based classifier (FC), to discriminate between treatment-experienced and treatment-naïve sequences. These classifiers were then evaluated on a smaller and phylogenetically very different African dataset (n = 3990) using the adjusted mutual information (panel **(a)**). This criterion measures how well the classifiers discriminate the two types of sequences (0: random classifier; 10° = 1: perfect classifier) and can be used to compute a p-value to see if the results are truly different to ones from a random classifier (an asterisk denotes a p-value ≤ 0.05). In panel (a), when all features are kept, both classifiers have high (close to 1), highly significant discriminatory power. In order to check where this power comes from, the authors did the same procedure, but this time removing features corresponding to known DRMs from the encoded vectors. The adjusted mutual information is lower than when using the full vectors, but still significantly better than random. Finally, the authors repeated the procedure after removing all sequences that had at least one known DRM from the training set. This time the adjusted mutual information indicates that the classifiers are no better than random. This shows that even after removing known DRMs from the data, there remains some resistance-associated information in the sequences, which differentiates treatment-naïve and treatment-experienced sequences. Furthermore, this information seems to be in the sequences that already contained DRMs, meaning that it most likely corresponds to accessory mutations that appear alongside known DRMs. By examining the LR and FC classifiers, we can extract the most important mutations in their decision-making; L228H and L228R of reverse transcriptase are two such mutations studied in Ref. [35•]. Site 228 in panel **(b)** is positioned right between the active site (where NRTIs act) and the regulatory 'NNIBP' site (where NNRTIs act). To check the accessory nature of these mutations, the authors computed relative risk between L228R/H and known DRMs. For a given known DRM, the relative risk corresponds to the prevalence of L228R/H in sequence that have that DRM, divided by the prevalence of L228R/H in sequences that do not have that DRM. In panels **(c.1)** and **(c.2)**, relative risks for L228R and L228H are shown with their 95% confidence intervals. These relative risks show that L228R and L228H are highly overrepresented both in sequences that contain DRMs to NRTI and NNRTI. This, as well as the physical proximity of site 228 to the sites where both classes of drugs operate, point to a potential role as accessory mutations to known DRMs.

Figure 2



Emergence and transmission of resistance in protease (mutation L90M, subtype B, UK).
Ancestral state reconstruction of the presence/absence of DRM L90M over time was performed and visualized by PastML [51•] on a phylogenetic tree inferred from 39 224 UK subtype B *pol* gene sequences [44] with RAxML-NG [52] and dated with LSD2 [60]. Resistance status was detected with sierrapy [23]. A sensitive resistance status for all tree nodes and tips before 1995 (year of acceptance of Saquinavir, the first antiretroviral therapy that can provoke L90M DRM) was imposed as in Ref. [61]. Circles denote clusters of samples with the same L90M state (green when the mutation is absent, orange for resistant strains); the sample sizes of clusters are indicated in the labels, for example, the circle 'resistant 119' represents the largest resistance cluster in 2016 (119 patients). Clusters with a '0' and two colors indicate internal tree nodes for which both resistant and sensitive states had similar marginal probabilities. Arrows between two circles denote transmissions from the top to the bottom cluster (i.e. acquired drug resistances correspond to the sensitive-to-resistant transmissions, while reversions correspond to the resistant-to-sensitive ones). The size and the number on top of the arrows indicate that the arrows represent multiple transmission events leading to clusters of similar sizes (e.g. the arrow of size 483 represents 483 acquired drug resistances). Overall, we see both a large number of independent acquisitions of drug resistance (arrows from green to orange circles), and the emergence of resistance clusters (orange circles of size >1). As expected, we do not see any resistance cluster in 1998, and small ones in 2005 (≤19 patients). We also see a substantial amount of reversions (e.g. 9 + 15 from the largest 2016 resistance cluster).

resistances (~83% in average, in UK subtype B [4]), while in resistance clusters we mostly observe naïve-to-naïve TDRs (~70% in average, in UK subtype B [4]). Reversions correspond to non-resistant tips and clusters descending from a resistance cluster. This approach is illustrated in Figure 2, where we used maximum-likelihood [52] to build a large tree containing 39 224 subtype B sequences from the UK, and infer [51•] the resistance status of all tree nodes for the L90M protease DRM. This mutation has a low fitness cost (see above), which likely explains its high frequency and high probability of transmission between treatment-naïve individuals, resulting in large resistance clusters and low reversion rate [4].

Next-generation sequencing, resistant minority variants

Standard population-based Sanger sequencing provides the genotypes of the predominant variants in a patient, but fails to detect resistant minority variants present in less than ~20% of the total viral population [53]. By contrast, next generation sequencing (NGS)-based pipelines not only lower sequencing costs, but also enable reliable and specific detection of resistant variants accounting for ~2% of the viral population [54,55•]. NGS is thus becoming the new standard for genotypic drug resistance testing for HIV [56,57•,58•,59•].

Resistant minority variants are suspected to cause virological failures that are difficult to predict using Sanger sequencing when their frequency is below 20%. In fact, the clinical impact of resistant minority variants is not uniform across drug classes and depends on the genetic barrier to resistance to specific drugs. NNRTIs in particular have a low genetic barrier (a single DNA mutation can drastically affect drug susceptibility) and many studies [62] have shown that resistant minority variants may

adversely affect the response to NNRTIs. Moreover, there is increasing evidence showing that resistant minority variants increase the risk of treatment switches and DRM accumulation [63]. All this, combined with the fact that NGS enables the quantification of DRM frequencies (and not solely their detection, as with Sanger sequencing), led to the development of many software pipelines to extract and quantify resistant minority variants from NGS data [55•]. For example, Hivmmer [64] is an alignment and variant-calling pipeline for Illumina HIV deep sequences, based on the probabilistic aligner Hmmer [65]. While the main pipelines are able to detect and quantify DRM frequencies [55•], there is still a need for standardization and quality assurance [57•]. Moreover, to our knowledge, no tool to predict resistance to major drugs of a representative sample of variants hosted by a patient exists for NGS data.

Resistant minority variants are also suspected to play a part in the transmission of DRMs. The study of a large international cohort of naïve patients using NGS resulted in the detection of a large fraction of DRMs corresponding to minority variants, which would not have been detected by traditional Sanger sequencing [59•]. Phylogenetic analyses [58•,66] indicate that some of these rare variants likely result from transmissions. However, careful analysis of resistance clusters favors the hypothesis that most resistant minority variants found in naïve patients are likely generated *de novo* as a result of replication errors [66].

Finally, new tools specifically designed for parsing the large volume of information contained within NGS datasets have recently begun to gain traction. For example, by simultaneously analyzing within-host and between-host pathogen sequences, phyloscanner [41] provides unprecedented resolution into the transmission process, allowing inference of the direction of transmission, the identification of TDRs and the detection of multiply infected individuals. Such an approach combined with rich NGS data and metadata should be of great help in phylodynamic studies [67•].

Perspectives

HIV drug resistance surveillance is essential to track TDR trends and shape first-line regimen recommendations, especially in low-income countries where DRMs are frequent, often multiple, and tend to increase [12,14,36,68]. We are at a crossroads where NGS should occupy a major place in HIV resistance surveillance and clinical care, thanks to its decreasing costs and ability to reveal resistant minority variants and study their impact. However, adoption of NGS-based HIV resistance genotyping poses pressing challenges [56,57•], especially for low-income countries, where they are most needed [58•,69]. In particular, there is a need for standardized analyses, validated pipelines, and public large-scale databases providing not only the within-host diversity of the virus at different time points, but also rich patient metadata (e.g. treatment history). In this context, machine learning and phylogenetic approaches are expected to play a major role, as they have already done with Sanger sequencing. Moreover, the use of modeling should increase to develop and monitor first-line and second-line treatment regimens [70•], and to characterize the impact of DRMs [71]. Lastly, the analysis of transmission networks [39,72–74] should help us gain further insight in HIV drug resistance surveillance.

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Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

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The 2019 edition of the IAS-USA drug resistance mutations list and Figure. The mutations listed are those that have been identified by specific criteria for evidence and drugs described. The Figure is designed to assist practitioners in identifying key mutations associated with resistance to antiretroviral drugs. This regularly updated material is useful for anyone working on HIVDR.

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The PANGEA (Phylogenetics and Networks for Generalized Epidemics in Africa) consortium has generated over 18 000 NGS HIV sequences from five countries in Eastern and Southern Africa: Botswana, Kenya, South Africa, Uganda and Zambia, sampled over 2014–2018. Combining phylogenetics, phylodynamics and epidemiology PANGEA-II will highlight where prevention efforts should be focused in sub-Saharan Africa to reduce the HIV epidemic most effectively. PANGEA offers accreditation to and welcomes project proposals and data contributions from external researchers who share their aims.

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This article presents recent advances in the application of deep learning to predict the resistance (phenotype) of HIV variants from their sequence (genotype). It utilizes publicly available HIV-1 sequence data and drug resistance assay results for 18 antiretroviral therapies to evaluate the performance of three architectures (multilayer perceptron, bidirectional recurrent neural network, and convolutional neural network). It identifies convolutional neural networks as the best performing architecture, with accuracy clearly superior to standard rule-based approaches, and distinguishes several sequence sites that are not known DRM locations and seem to be associated to resistance.

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This article uses machine learning to discover new DRMs and study potential epistasis effects. It applies this approach to a very large UK dataset comprising ≈55 000 reverse transcriptase sequences. Results robustness is checked on different UK and African datasets. Six new mutations associated to resistance are found. All six have a low genetic barrier and show high correlations with known DRMs. Importantly, the results indicate that epistasis seems to be limited to the classical scheme where primary DRMs confer resistance and associated mutations modulate the strength of the resistance and/or compensate for the fitness cost induced by DRMs.

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PastML is a fast maximum likelihood method and tool for ancestral scenario reconstruction and visualization on phylogenetic trees. It uses decision-theory concepts to associate each node in the tree to a set of likely states. Its application to HIV1-C data set inferred many cases of independent emergence of resistance mutations under treatment pressure, and detected few resistance clusters, corresponding to transmissions among untreated patients.

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Many NGS HIVDR data analysis pipelines have been independently developed, each with variable outputs and data management protocols. This article compares the performance of five NGS HIVDR pipelines (HyDRA, MiCall, PASeq, Hivmmer and DEEPGEN) using proficiency panel samples from NIAID Virology Quality Assurance (VQA) program. All pipelines detect amino acid variants (AAV) at full range of frequencies (1–100%) and demonstrate good linearity as compared to the reference frequency values. However, their specificity dramatically decreases at AAV frequencies <2%, suggesting that 2% threshold may be a more reliable reporting threshold for ensured specificity in AAV calling and reporting. Findings from this study highlight the need for standardized strategies for NGS HIVDR data analysis.

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Several challenges still exist for the standardization and quality assurance of NGS-based HIVDR genotyping. This article highlight considerations of these challenges as related to laboratory, clinical, and implementation of NGS for HIV drug resistance testing. Several sources of variation and bias

occur in each step of the general NGS workflow, that is, starting material, sample type, PCR amplification, library preparation method, instrument and sequencing chemistry-inherent errors, and data analysis options and limitations. Additionally, adoption of NGS-based HIVDR genotyping, especially for clinical care, poses pressing challenges, especially for resource-poor settings, including infrastructure and equipment requirements and cost, logistic and supply chains, instrument service availability, personnel training, validated laboratory protocols, and standardized analysis outputs.

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Despite decreasing costs, next-generation sequencing (NGS) is still prohibitively costly for routine use in generalized HIV epidemics in low-income and middle-income countries. This article present veSEQ-HIV, a high-throughput, cost-effective NGS sequencing method and computational pipeline tailored specifically to HIV, which can be performed using leftover blood drawn for routine CD4 cell count testing. This method overcomes several major technical challenges that have prevented HIV sequencing from being used routinely in public health efforts. The complete veSEQ-HIV pipeline provides viral load estimates and quantitative summaries of drug resistance mutations; using phyloscanner software [41], it also exploits information on within-host viral diversity to construct directed transmission networks.

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This analysis characterized TDR in Strategic Timing of Antiretroviral Treatment (START) study participants by NGS. START enrolled ~5000 antiretroviral therapy naïve individuals in 35 countries between 2009 and 2013, out of which for ~3000 participants baseline NGS data at study entry were available. The study used statistical methods to analyze the WHO 2009 surveillance DRMS, as well as reverse transcriptase mutations T215N and E138K, and INSTI surveillance mutations from Stanford HIVdb, using three thresholds: > 2%, > 5% and >20% of the viral population. A large proportion of low-level variants was detected, which would not have been possible with traditional Sanger sequencing.

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The study uses phyloscanner software [41] on deep-sequencing data from a large population-based sample of HIV-infected individuals in Rakai District, Uganda [21] to investigate the sources of epidemic spread by reconstructing partial transmission networks, and inferring the direction of transmission.

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The MARISA model presented in this article aims at investigating the time trends and factors driving NNRTI resistance in South Africa. MARISA is a compartmental model that includes the key aspects of the local HIV epidemic: continuum of care, disease progression, and gender. The dynamics of NNRTI resistance emergence and transmission are then added to this framework. Using this novel approach of triangulating clinical and resistance data from various sources, MARISA reproduces the time trends of HIV in South Africa in 2005–2016, with a decrease in new infections, undiagnosed individuals, and AIDS-related deaths. MARISA also captures the dynamics of the spread of NNRTI resistance: high levels of acquired drug resistance, and increasing transmitted drug resistance. MARISA shows that rapid antiretroviral therapy scale-up and inadequate switching to second-line treatment were the key drivers of the spread of NNRTI resistance in South Africa. These results highlight the need of alternative first-line regimens in this region.

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